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BOTULISM IN JUVENILE COHO SALMON (*ONCORHYNCHUS KISUTCH*) IN THE UNITED STATES

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ABSTRACT

Eklund, M.W., Peterson, M.E., Poysky, F.T., Peck, L.W. and Conrad, J.F., 1982. Botulism in juvenile coho salmon (*Oncorhynchus kisutch*) in the United States. *Aquaculture*, 27: 1—11.

Botulism type E was first recognized as a major cause of fish mortality in the United States in 1979. This disease caused an estimated loss of 1¼ million juvenile salmon reared in earth-bottom ponds during the summer and fall of 1979 and 1980. The botulism outbreaks, preliminary laboratory results of experimental botulism, and precautions to be taken by fish farm employees are discussed.

INTRODUCTION

Botulism is a neuroparalytic disease that is frequently lethal to man and animals. Foodborne, infant, and wound botulism are the three clinical forms that are currently recognized. Foodborne botulism is caused by the ingestion of toxin produced by the bacteria *Clostridium botulinum* during its growth in feeds and foods. Infant and wound botulism are associated with the growth and toxin production of the organism in the intestines or in damaged tissue.

Based upon the production of antigenically specific neurotoxins, the species *C. botulinum* is divided into different types designated by the letters A through G. The distribution of these toxin types varies with the geographical area, but in general type E is the most prevalent in marine and freshwater environments of the Northern Hemisphere. All of the toxins produced by the different types are lethal, but the sensitivity of man and different animal species varies with the *C. botulinum* toxin type.

Botulism or "bankrupt disease" was first reported in pond-reared trout in Denmark by Huss and Eskildsen in 1974. The source of the toxin in these

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outbreaks was from the growth of *C. botulinum* type E in feeds stored at nonrefrigerated temperatures and from cannibalism of dead fish accumulating in the bottom of the rearing ponds.

This report describes the first recognized type E botulism outbreak in pond-reared fish in the United States. These outbreaks occurred during the fall of 1979 and 1980 and resulted in the loss of over 1¼ million juvenile coho salmon (*Oncorhynchus kisutch*) in Washington and Oregon State Hatcheries. The diagnosis of the disease, multiplication of *C. botulinum* type E in pond sediments, preliminary laboratory experiments of botulism in juvenile salmonids, and precautions to be taken by hatchery employees are also discussed.

MATERIALS AND METHODS

Botulinum toxin assay and neutralization tests

The flesh or intestines of fish dying with botulism symptoms were ground and extracted with 2 ml of physiological saline or gelatin-phosphate buffer (Duff et al., 1956). After extracting for 1 h, the samples were centrifuged and 0.5 ml of supernatant fluid or fluid treated with trypsin (Duff et al., 1956) was injected intraperitoneally (I.P.) into Swiss Webster mice weighing 18 to 26 g. Toxin titrations were made by diluting samples in log intervals in gelatin-phosphate buffer and injecting mice I.P. with 0.5 ml of each dilution. Sediment samples from the rearing ponds were centrifuged and supernatant fluids were assayed for toxin before and after trypsin treatment. Water from the hatchery ponds was concentrated by dialyzing against polyethylene glycol (Kahn, 1959) and assayed for toxin. Toxin neutralization tests were made using the mouse protection test and monovalent *C. botulinum* antiserum as outlined by Eklund and Poysky (1972).

Isolation and enumeration of C. botulinum type E from fish and sediments

TPG medium (5% trypticase, 0.5% peptone, and 0.4% glucose) containing a final concentration of 0.1% sodium thioglycollate was used for culturing type E organisms. Aliquots of fish intestines, sediments, or their enrichment cultures were streaked onto egg yolk agar plates. Following incubation, typical isolated colonies displaying irridescence were selected and picked into TPG broth and and tested for toxicity after 3 days of incubation at 30° C. Type E organisms were enumerated in sediment samples using the TPG medium and the three-tube Most Probable Number procedure. The presence of *C. botulinum* type E toxin was used as confirmation of type E growth.

Sensitivity of fish to C. botulinum type E toxin

Type E toxin was produced in TPG medium at 30° C using isolates from

the hatchery sediments or the toxin was extracted from the flesh or intestines of fish displaying botulism symptoms. Fish weighing 8 to 12 g were tested for their sensitivity to untreated and trypsin-treated type E toxin by the intraperitoneal and oral routes.

RESULTS AND DISCUSSION

Mortality in salmon hatcheries and confirmation of botulism

This report discusses two botulism outbreaks at the Washington State Elokomin Hatchery in 1979 and 1980 and one at the Oregon State Klaskanine Hatchery in 1980.

Outbreak at Elokomin in 1979

Pond 23 is a 0.53-acre earth-bottom pond with an average water depth of 3.5 ft and a maximum depth of 5 ft near the outlet. It has an inflow of water of 17 ft^3/s. In May 1979, Pond 23 was cleaned and repopulated with approximately two million coho salmon weighing 1 to 2 g each.

On 28 August, the fish began to be hypersensitive and nervous, and within a 3-day period, losses increased from 20 to 800 fish per day and remained at this level for approximately 1 week (Fig. 1). The mortality rate then began to double each day until the losses reached 70 000 fish per day. On 21 September, the majority of the survivors were moved to another earth pond designated Number 22. The fish remaining in Pond 23 continued to get botulism and within a 3-week period there were no survivors. The mortality rate in Pond 22 decreased to 2000 fish per day for a 2-week period and then suddenly increased again to 22 000 fish (Fig. 1). The daily losses remained in the range of 16 000 to 19 000 for an additional 4 days; then decreased to 2000 fish per day and continued at this level until the first part of November when the outbreak stopped. This outbreak resulted in a total loss of approximately one million fish.

Fish with botulism appeared to lose their equilibrium and would swim on one side and then on the other. They were unable to swim against the water current and, as a result, were forced to the outlet screen or to low flow areas where they would lie on their sides. When disturbed, they would move toward the surface in a jerking motion only to sink again to the bottom as though they were tail-heavy. Once the fish developed symptoms, death was inevitable. The symptoms persisted for many hours in some fish, whereas in others death occurred rapidly. The water temperature of the ponds ranged from 14 to 16.6° C during the months of July through the middle of October while the outbreak was in progress. During the latter part of October when the botulism outbreak stopped, the water temperatures decreased to temperatures in the range of 10.5 to 14.5° C.

The cause of the outbreak was determined during the middle of October

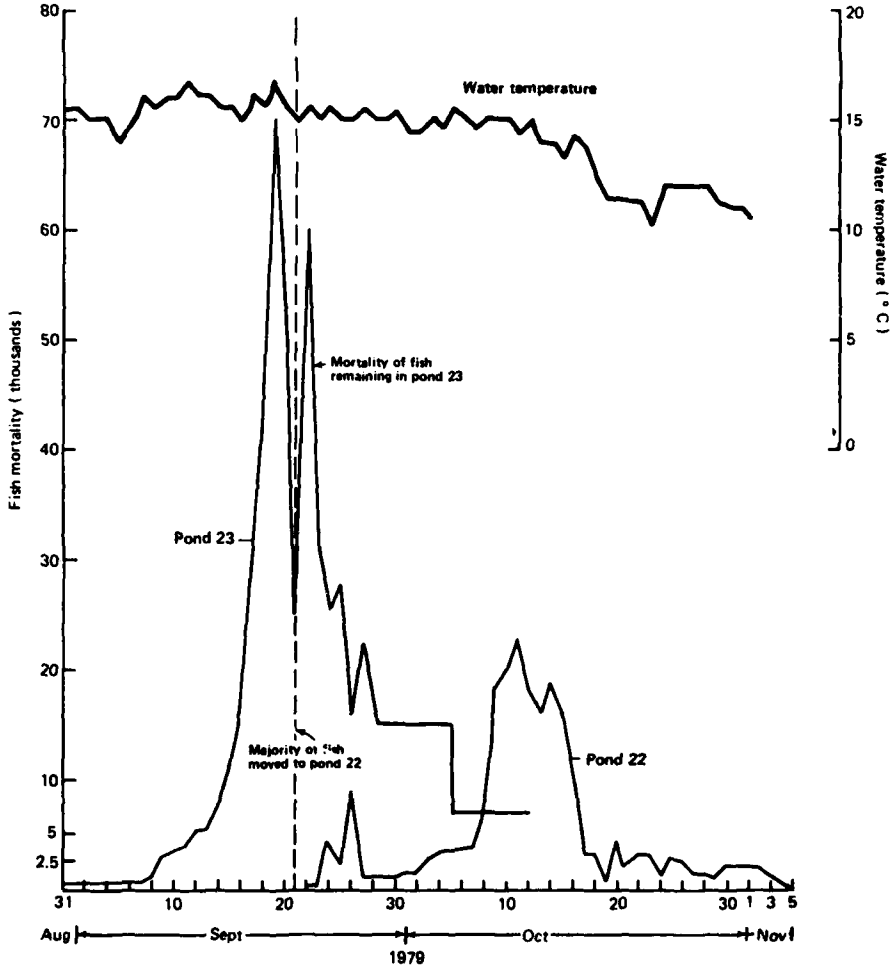

Fig. 1. Fish mortality from botulism outbreak at Elokomin Hatchery in 1979. After 28 September, the fish mortality in Pond 23 is reported as the daily average per week.

when *C. botulinum* type E toxin was demonstrated in the stomach and intestinal contents and flesh of morbid fish. The last 10—15 mm of the intestinal tract contained very viscous yellow-orange fecal material. This suggested a metabolic disorder of the digestive system or a state of constipation, a sympton frequently observed in animal or human botulism. No other pathogenic conditions were observed.

When the intestines of the morbid fish were ground and extracted with gelatin-phosphate buffer, many of the supernatant fluids of the extracts

produced characteristic botulism symptoms in mice and in juvenile salmon when the toxin was introduced by the I.P. route. When toxic extracts were mixed with *C. botulinum* type E antitoxin, they were no longer lethal to either mice or fish. Antitoxins from other *C. botulinum* types, however, did not offer this same protection. This specificity of neutralization confirmed the presence of *C. botulinum* type E toxin in the fish intestines and flesh.

Typical results of the toxin assays from morbid fish are summarized in Table I. The titers of the intestinal contents ranged from 2 to 200 MLD per

TABLE I

Detection of type E toxin in intestines of fish with botulism symptoms

Date collected	No. toxic/no. tested	Range of toxin titers[a]
5 October	2/9	2—200
9 October	5/5	2—200
9 October	2/10	2—100
16 October	16/20	2—200
18 October	3/6	2—20

[a] Fish containing 100 MLD of toxin per ml of intestinal extract frequently contained 20 MLD of toxin per g of flesh.

ml of extract or a total of 6 to 600 MLD per fish stomach and intestines. When fish intestines contained greater than 100 MLD toxin per ml, 20 MLD of toxin was often found in the flesh. In some cases, toxin was detectable in the flesh but not detectable in the intestines. Trypsin treatment increased the toxin titers of some intestinal samples, but did not increase the titers of the extracts from the fish flesh.

The bacterium *C. botulinum* type E was isolated numerous times from the intestines of the fish or from pond sediments which contained 75 000 type E organisms per g. The toxin from the type E culture filtrates was lethal to fish by the oral and intraperitoneal routes and produced botulism symptoms identical to those observed in the botulism outbreaks at the hatcheries. Type E antitoxin again protected the fish from the lethal toxin.

Outbreak at Elokomin Hatchery in 1980

Following the 1979 botulism outbreak, the sediments of Pond 23 were removed and the pond was relined with new gravel. The pond was repopulated with one million coho salmon weighing 1 to 2 g each in December 1979. Starting in March, sediment samples from Pond 23 were collected and *C. botulinum* type E populations determined. The first samples contained 400 type E organisms per g (Table II). The population increased to 24 000 by May and then remained relatively constant for the next 2 months. In

TABLE II

Growth of *C. botulinum* type E in sediments of Pond 23 in 1980

Month	Water temperature (°C) Range	Mean	Type E organisms per g sediment
March	5.0— 8.3	7.2	400
April	6.1—12.8	9.4	1500
May	7.2—10.6	10.6	24000
July	11.1—20.0	15.6	15000
August	6.1—18.3	15.6	15000
15 September	5.5—15.6	13.3	46000
24 September	10.6—13.3	12.8	240000
October	6.1—14.4	10.6	150000

September, the number of type E organisms increased to 46 000 and continued to increase until some of the sediment samples contained 240 000 per g. The highest population of type E occurred within 20 ft of the outlet screen where waste feed, fecal material, dead fish, and other sediments accumulated to depths of 4 to 6 inches. Lower populations of type E (110 to 4600 per g) were present in other areas of the pond where the accumulation of sediments was 1 to 2 inches deep. The water temperatures of the pond started at 5.0 to 8.3°C in March, increased to a high of 20°C in July, and then decreased thereafter. Increases in type E populations did not appear to be correlated with the water temperatures in the pond.

The fish mortality during the period of December 1979 to September 1980 remained at less than 20 fish per day. The same mortality pattern observed in 1979, however, began to reappear during the first 2 weeks in September 1980. Fig. 2 shows the daily losses increasing from 20 to 400 per

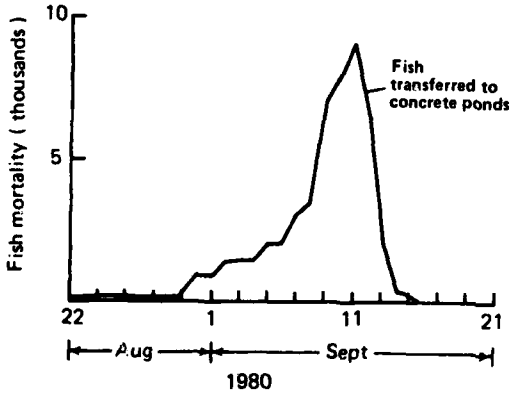

Fig. 2. Fish mortality from botulism outbreak at Elokomin Hatchery in 1980.

day during the latter part of August and then doubling each day until the losses reached 9000 fish. Based upon the increases in type E populations in the sediments and the previous year's experience with botulism, the fish were transferred to concrete ponds and the mortality began to decrease immediately. Within 5 days, the fish losses were less than 100 per day. No further problems were encountered with this fish population.

Klaskanine Hatchery outbreak in 1980

The earth bottom pond referred to as the Lake at the Klaskanine Hatchery is of comparable size and has about the same water flow as Pond 23 at the Elokomin Hatchery. The Lake has had epizootics of unknown etiology since 1960. The causative agent of the outbreaks was not known until 1980 when we demonstrated type E toxin in the digestive tract and flesh of morbid fish. This outbreak resulted in losses of 260 000 yearling coho salmon out of an original population of 871 000 fish.

The mortality pattern was similar to that observed at the Elokomin Hatchery with the exception that the outbreak was somewhat self-limiting. The losses started to increase on 14 September from 20 to over 200 fish per day. For 2 weeks, the mortality remained at 800 fish per day, and then continued to increase until the daily loss was 24 000 fish. After that, the losses steadily decreased and within 2 more weeks, the outbreak stopped (Fig. 3). The Lake sediments contained smaller populations of type E, 2400 to 11 000 organisms per g, than the Elokomin Pond 23. In addition, Lake sediments contained other bacteria which inactivated type E toxin when sediments were cultured in TPG medium. When sediments from the Elokomin pond were cultured, this inactivation of toxin was not observed. These microbial differences could have contributed to the magnitude and duration of the outbreaks at the two hatcheries. The water temperature of the Lake ranged from 12 to 15.5°C during the month of September and the early part of October and decreased during the latter phases of the outbreak.

PRELIMINARY LABORATORY EXPERIMENTS AND SOURCE OF TOXIN

When fish were force fed capsules containing 0.1 ml of different titers of filter-sterilized type E toxin, the minimum lethal dose for a 10-g fish held in 15°C water was 200 MLD (based upon trypsinized mouse intraperitoneal toxin titers). Immediately following the development of botulism symptoms and death, the digestive tracts of these fish were extracted and assayed for unabsorbed toxin. A toxin titer of 400 MLD was detected in the stomach and intestines of the fish if they had been fed 2000 MLD of toxin. In comparison, fish dying of botulism in the hatcheries often contained 6 to 600 MLD of type E toxin in their digestive tracts. Some of these hatchery fish therefore were exposed either to high concentrations of toxin, or to a continuous source of toxin, or the type E organism grew and produced toxin in the in-

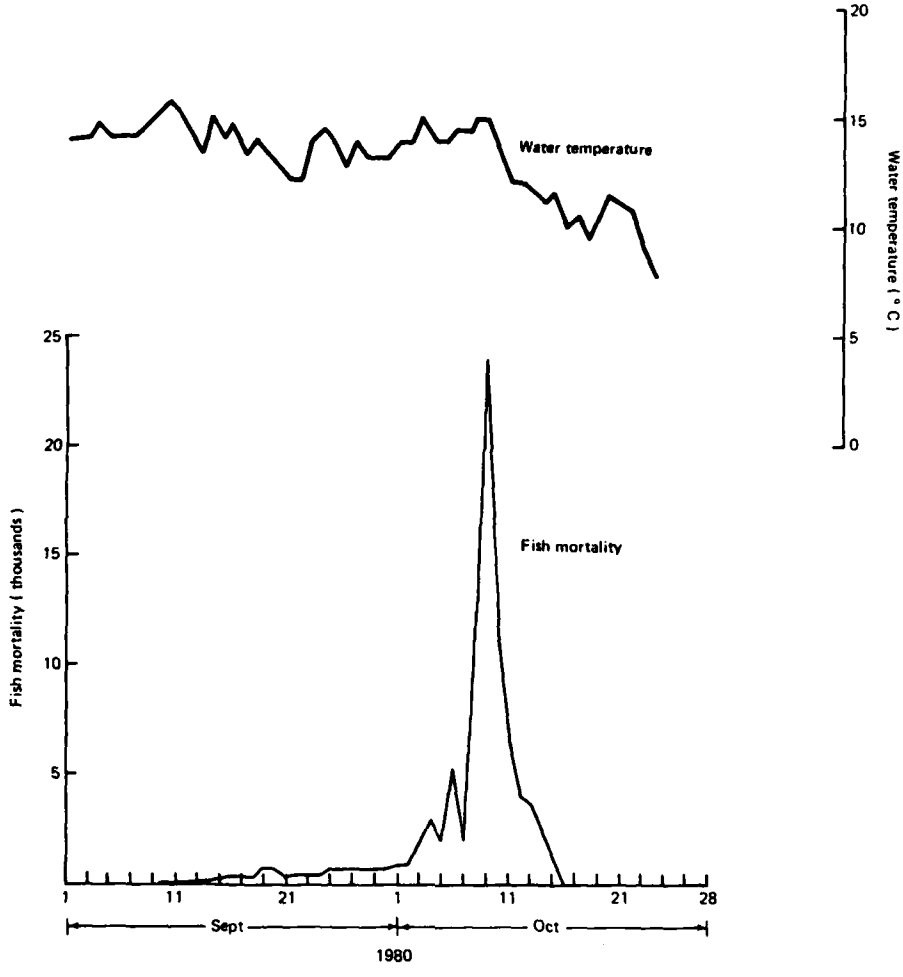

Fig. 3. Fish mortality from botulism outbreak at Klaskanine Hatchery in 1980.

testines of some of the fish in a manner analogous to the development of infant botulism in humans. The fish feed and different samples collected from the hatchery ponds were therefore assayed to determine the source of the type E toxin. Oregon moist pellets were used by both hatcheries and were stored in freezers until they were used. Neither toxin nor viable *C. botulinum* organisms were detected in these pellets. Water samples were collected at different times, but toxin was not detectable even after they had been concentrated 60 times by dialyzing against polyethylene glycol. Toxin was not present in detectable concentrations in any of the 93 pond sediment samples collected at the Elokomin Hatchery, but 2 MLD of type E toxin

was demonstrated in one of the 16 samples collected at the Klaskanine Hatchery. Large numbers of tubifex worms were frequently present in the sediments from both hatcheries, but only one of the 28 tubifex samples contained type E toxin (2 MLD per g) which was detectable only after trypsin activation.

Dead fish accumulating on the pond bottoms were the greatest source of toxin often containing 200 MLD of toxin per g. This undoubtedly was the combined effect of the toxin consumed by the fish prior to death and the subsequent growth and toxin production by *C. botulinum* type E in the dead fish. Cannibalism was not evident in the fish that had been dead for 1 or 2 days, but could have occurred in fish that had been dead for longer periods of time and where advanced decomposition obscured signs of cannibalism.

We concur with Huss and Eskildsen's (1974) recommendations that dead fish undergoing decomposition are a potential source of toxin and therefore should not be permitted to accumulate in the pond bottoms. Because of the depths and cloudiness of the water in some ponds, this practice, however, often cannot be followed. Controlled experiments therefore are currently in progress to determine a method for controlling the growth of type E in the sediments, which would also eliminate sources of low titered toxins in the sediments and tubifex worms.

PRECAUTIONS FOR FISH FARM EMPLOYEES

C. botulinum bacteria produce the most potent toxin known. Because of the dangers of this toxin to man and animals, extreme precautions should be taken by all fish farm and hatchery personnel. The dead fish should be incinerated or placed in trenches away from domestic water supplies and buried under a layer of quick lime and soil. Otherwise, the fish botulism outbreaks could be extended to domesticated or wild bird and animal populations.

Employees should be informed that the *C. botulinum* organism and its toxin are potentially dangerous to themselves and their families. Great care therefore should be exercised when employees work with dead fish as the feces and flesh will often contain toxin which could be absorbed through cuts or even taken internally if improper hygiene were followed.

Type E bacteria could potentially grow and produce wound botulism (Dezfulian and Dowell, 1980) if it is introduced into damaged tissue of humans. In addition, children under 1½ years of age also can develop infant botulism by the growth of the *C. botulinum* bacteria in the intestines. *C. botulinum* types A, B, and F are the only types that have caused infant botulism since it was first discovered in 1975 (Arnon et al., 1977), but the potential of type E infant botulism exists.

C. botulinum type E has several characteristics which increase its dangers to humans in the form of food poisoning. It can grow and produce toxin at temperatures as low as 3.3°C and, because of its nonproteolytic characteris-

tics, its growth in foods cannot be detected by off-odors and off-flavors. If type E is brought into the household, especially in high numbers, through contaminated clothing or improperly washed hands, it could be introduced into cooked or uncooked meats, fish, and vegetables. Toxin can be produced in these contaminated foods within 24 h at room temperature, 1 week at 10° C, and 3 weeks at 3.3° C. Consumption of these foods therefore could result in fatal forms of botulism-food poisoning. It is important that good sanitation and food handling practices be used in the home at all times. Perishable foods should be stored below 3.3° C and consumed within a fe days. For longer storage, the foods should be frozen. Freezing does not destroy the organism or its toxin, but it will stop *C. botulinum* from gr and producing toxin. If canning, pickling, smoking, drying, or other me are used to preserve foods in the home, approved recommended preserv procedures should be followed.

It is therefore essential that employees wear protective clothing and whenever working with the diseased fish and that good personal hygiene followed. Antiserum against the *C. botulinum* toxins is available for humans, but its effectiveness in controlling botulism depends upon the advancement of the disease. Because of the extreme toxicity of botulinal toxins, prevention of the disease is recommended.

The sediments of the ponds in which botulism has occurred contain unusually large numbers of *C. botulinum* type E organisms. These sediments should not be used as fertilizer for home vegetable or flower gardens or for other purposes. Instead the sediments should be buried in the same manner as recommended for dead fish.

Botulism has only been demonstrated in small fish from hatcheries. If botulism is demonstrated in fish of marketable size, it is very important that during the outbreak none of the fish, healthy or morbid, be consumed by man or animals as botulism symptoms could be advancing in the fish at different rates, and the flesh could contain lethal levels of toxin.

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